Suborganelle Sensing of Mitochondrial cAMP-Dependent Protein Kinase Activity

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Supporting Information

Table S1. Library of quencher dyes. Phosphorylation-induced fluorescence fold changes of peptide **1** in the presence of the quencher dyes (non-optimized conditions).

Dye	Name	Fold Change
D1	Acid Green 27	3.95
D2	Acid Blue 40	1.36
D3	Evans Blue	1.88
D4	Acid Alizarin Violet N	1.53
D5	Acid Blue 80	1.57
D6	Reactive Blue 2	2.51
D7	N,N-dimethylnitrosoaniline	1.21
D8	Cresol Red	1.47
D9	Phenol Red	1.2
D10	Methyl Orange	1.18
D11	Bromophenol Blue	1.49
D12	BUFFER	1.16
D13	Xylene Cyanol FF	1.15
D14	Disperse Yellow 3	1.15
D15	Ethyl Orange	1.27
D16	Methylene Blue	1.14
D17	Brilliant Blue R	1.16
D18	Eriochrome Black T	2.04
D19	Alizarin Red	1.31
D20	Malachite Green oxalate	1.16
D21	Phenolphthalein	1.17
D22	Carminic Acid	1.15
D23	Nuclear Fast Red	1.16
D24	Acid Fuchsin	1.18
D25	Acridine Orange	1.17
D26	Acridine Yellow G	1.11
D27	Aniline Blue WS	1.2
D28	Azure A	1.13
D29	Azure B bromide	1.12
D30	Basic Fuchsin	1.17
D31	Bismark Brown Y	1.17
D32	Brilliant Yellow	1.65
D33	Bromocresol Purple	2.33
D34	Chlorazol Black E	1.72
D35	Chlorophenol Red	1.2
D36	Chrysoidine Y	1.16
D37	Erythrosin	1.17
D38	Ethyl Violet	1.14

D39	Naphthol Blue Black	2.27
D40	Methylthymol Blue	1.31
D41	Methyl Violet	1.14
D42	Ponceau S	1.24
D43	Rose Bengal	1.2
D44	Rosolic Acid	1.16
D45	Safranin O	1.18
D46	Serva Violet 49	1.16
D47	Tartrazine	1.18
D48	Trypan Blue	1.55

Figure S1. ATP(γ)S serves as a weak ATP analog in the PKA-catalyzed thiophosphorylation of peptide **1**. Experiments were performed with 10 μM 14-3-3, 5 mM $MgCl_2$, 2 mM DTT, 50 mM Tris HCl at pH 7.5, 1 μM peptide **1**, and 30 μM **4** in the presence of 1 mM ATP or 1 mM ATP(γ)S. 0.5 nM PKA was added after 100 s and the reaction progress was monitored and plotted as fold change verses time. The kinase reaction was significantly slower with ATP(γ)S, however the peptide was thiophosphorylated under these conditions. Mass found m/z 1673.2 (calculated m/z 1673.8).

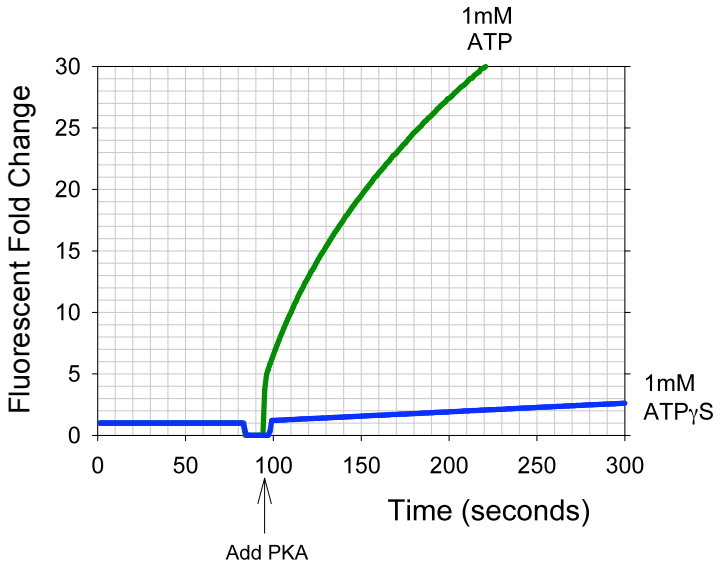

Figure S2. Fluorescence fold-change as a function of time in the absence (0 μM) and presence of 14-3-3. Experiments were performed with 5 mM $MgCl_2$, 2 mM DTT, 50 mM Tris HCl at pH 7.5, 1 μM peptide, 30 μM **4**, 1 mM ATP, and varying concentrations of 14-3-3. 0.5 nM PKA was added at 100 s to initiate the reaction.

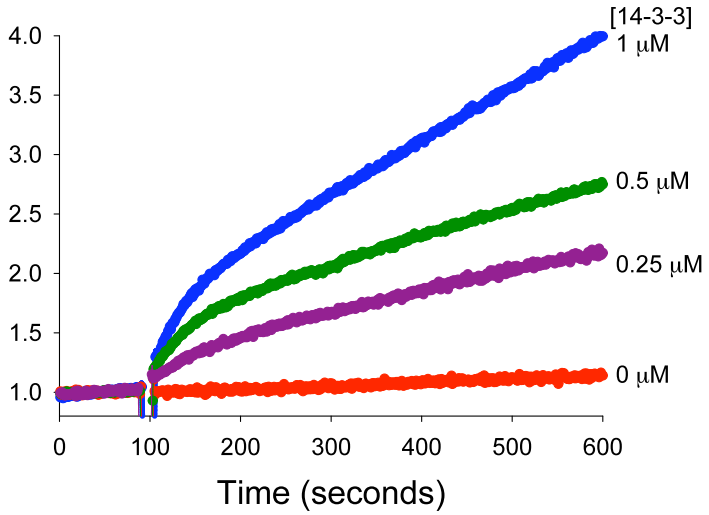

Figure S3. Fluorescence fold-change as a function of time in the absence of fluorescent quencher **4.** Experiments were performed with 10 μM 14-3-3, 5 mM $MgCl_2$, 2 mM DTT, 50 mM Tris HCl at pH 7.5, 1 μM peptide **1**, 1 mM ATP, and two different concentrations of PKA [1 nM (blue curve) and 2 nM (red curve)] to initiate the reaction.

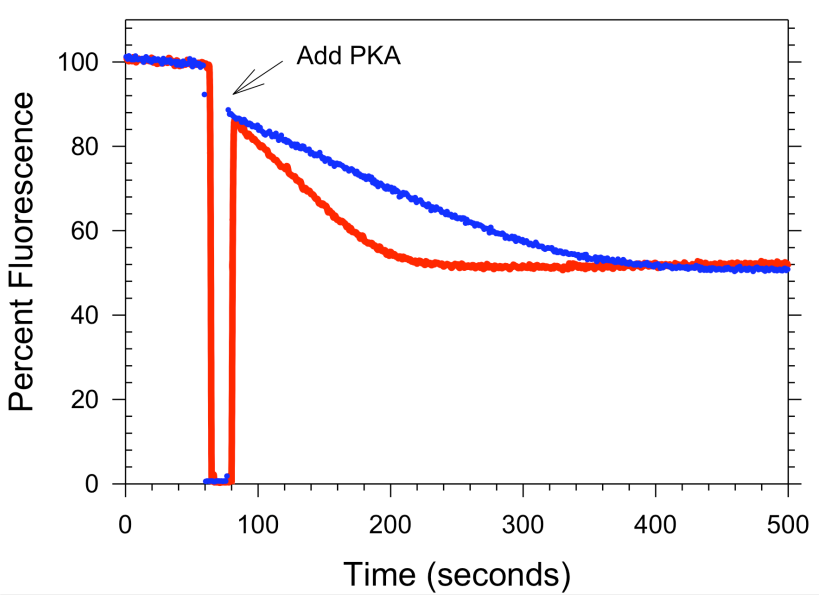

Figure S4. 3-dimensional structure of a 14-3-3 domain/phosphopeptide complex**.** The tryptophan residues of the 14-3-3 domain are highlighted in lime green, whereas the remainder of the 14-3-3 domain is shown as a grey cartoon. The N-terminus of the phosphopeptide is indicated with an arrow. Coordinates were downloaded from the Protein Data Bank (PDB ID 1QJA).

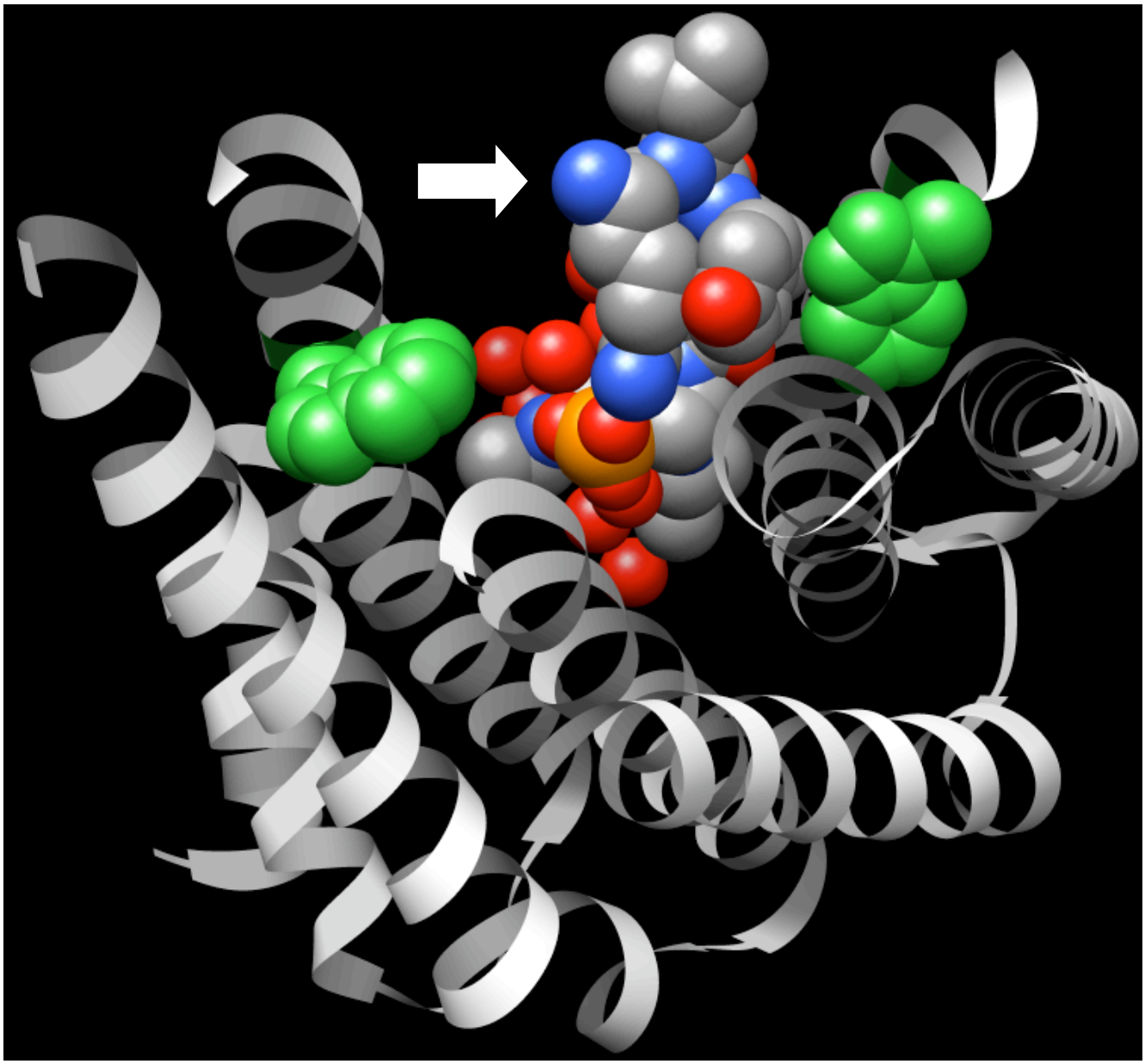

Figure S5. Mitochondrial-driven fluorescence enhancement is cAMP-dependent. Experiments were performed using 1 mM ATP, 5 mM $MgCl_2$, 2 mM DTT in 50 mM Tris HCl at pH 7.5, 1 μM peptide sensor **1**, 30 μM **4**, and 10 μM 14-3-3 incubated for 2 min, after which time 0.22 mg/mL of total mitochondrial lysate was added. After incubating for another 2 min, 1 mM cAMP was added. The reaction progress was monitored and plotted as fluorescence intensity versus time.

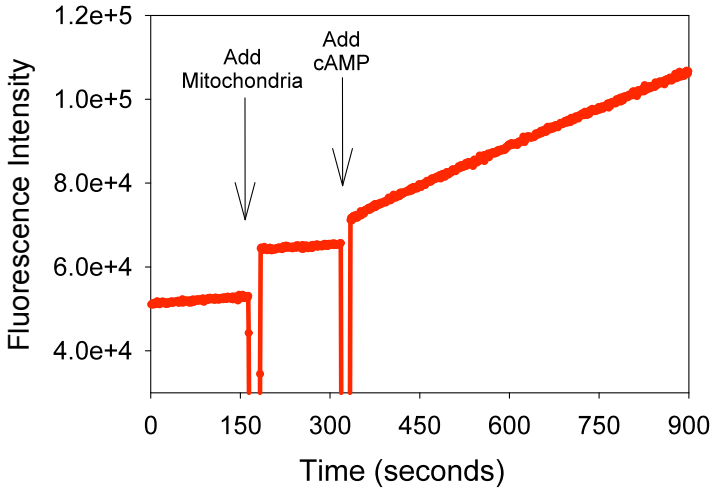

Figure S6. The PKA inhibitor H-89 blocks the fluorescent enhancement driven by (A) the C subunit (IC_{50} = 26 ± 9 μM) and (B) by cAMP-treated mitochondria. The experiments were performed under standard conditions (1 mM ATP, 5 mM $MgCl_2$, 2 mM DTT, 1 μM peptide **1**,10 μM 14-3-3, 30 μM **4**, 50mM Tris HCl at pH 7.5). For the experiments shown in (B), mitochondria were added after a 2 min pre-incubation. H-89 was subsequently introduced following a second 2 min incubation.